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What is claimed is:

1. A method for preparing liposomes containing at least one biologically active substance encapsulated therein under mild conditions, said method comprising the following steps:

- 5 (A) providing liposomes, wherein the liposomes are prepared by a method other than the instant method;
- (B) mixing the product of step (A) with aqueous medium U and a water-miscible organic solvent to form a gel or a liquid containing gel particles; and thereafter
- 10 (C) (a) mixing the gel or liquid containing gel particles with aqueous medium V to directly form the liposomes containing the at least one biologically active substance encapsulated therein,
- (b) (i) mixing the gel or liquid containing gel particles with aqueous medium V to form a curd or curdy substance; and
- 15 (ii) mixing the curd or curdy substance with aqueous medium W to directly form the liposomes containing the at least one biologically active substance encapsulated therein, or
- (c) (i) cooling the gel or liquid containing gel particles to form a waxy substance; and
- 20 (ii) mixing the waxy substance with aqueous medium W to directly form the liposomes containing the at least one biologically active substance encapsulated therein;

 wherein the at least one biologically active substance is added in step (A), step (B) and/or step (C), and wherein aqueous media U, V and W are the same or

25 different.

2. A method for preparing liposomes containing at least one biologically active substance encapsulated therein under mild conditions, said method comprising the following steps:

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(A) (a) (i) providing liposomes, wherein the liposomes are prepared by a method other than the instant method; and

(ii) mixing the liposomes of step (A)(a)(i) with the at least one biologically active substance;

5 (b) (i) providing liposomes in aqueous medium U, wherein the liposomes are prepared by a method other than the instant method; and

(ii) mixing the liposomes of step (A)(b)(i) with the at least one biologically active substance;

10 (c) (i) providing liposomes, wherein the liposomes are prepared by a method other than the instant method; and

(ii) mixing the liposomes of step (A)(c)(i) with aqueous medium U and the at least one biologically active substance;

(d) (i) providing liposomes in aqueous medium U, wherein the liposomes are prepared by a method other than the instant method; and

15 (ii) mixing the liposomes of step (A)(d)(i) with aqueous medium U and the at least one biologically active substance;

(e) forming liposomes in the presence of the at least one biologically active substance by a method other than the instant method;

20 (f) providing liposomes containing the at least one biologically active substance, wherein the liposomes are prepared by a method other than the instant method; or

(g) providing liposomes, wherein the liposomes are prepared by a method other than the instant method;

25 (B) (a) mixing the product of step (A)(b), (A)(c) or (A)(d) with a water-miscible organic solvent to form a gel or a liquid containing gel particles;

(b) mixing the product of step (A)(a), (A)(e) or (A)(f) with aqueous medium U and a water-miscible organic solvent to form a gel or a liquid containing gel particles;

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(c) mixing the product of step (A)(g) with aqueous medium U, a water-miscible organic solvent and the at least one biologically active substance to form a gel or a liquid containing gel particles; or

5 (d) mixing the product of step (A)(g) with aqueous medium U and a water-miscible organic solvent to form a gel or a liquid containing gel particles;
and thereafter

(C) (a) mixing the gel or liquid containing gel particles of step (B)(a), (B)(b) or (B)(c) with aqueous medium V to directly form the liposomes containing the at least one biologically active substance encapsulated therein,

10 (b) (i) mixing the gel or liquid containing gel particles of step (B)(a), (B)(b) or (B)(c) with aqueous medium V to form a curd or curdy substance; and

(ii) mixing the curd or curdy substance with aqueous
15 medium W to directly form the liposomes containing the at least one biologically active substance encapsulated therein;

(c) (i) cooling the gel or liquid containing gel particles of step (B)(a), (B)(b) or (B)(c) to form a waxy substance;

(ii) mixing the waxy substance with aqueous medium W
20 to directly form the liposomes containing the at least one biologically active substance encapsulated therein;

(d) mixing the gel or liquid containing gel particles of step (B)(d) with aqueous medium V and the at least one biologically active substance to directly form the liposomes containing the at least one biologically active
25 substance encapsulated therein,

(e) (i) mixing the gel or liquid containing gel particles of step (B)(d) with aqueous medium V and the at least one biologically active substance to form a curd or curdy substance; and

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(ii) mixing the curd or curdy substance with aqueous medium W to directly form the liposomes containing the at least one biologically active substance encapsulated therein;

5 (f) (i) mixing the gel or liquid containing gel particles of step (B)(d) with aqueous medium V to form a curd or curdy substance; and

(ii) mixing the curd or curdy substance with aqueous medium W and the at least one biologically active substance to directly form the liposomes containing the at least one biologically active substance encapsulated therein; or

10 (g) (i) cooling the gel or liquid containing gel particles of step (B)(d) to form a waxy substance;

(ii) mixing the waxy substance with aqueous medium W and the at least one biologically active substance to directly form the liposomes containing the at least one biologically active substance encapsulated therein;

15 wherein aqueous media U, V and W are the same or different.

3. The method of claim 2, wherein the liposomes containing the at least one biologically active substance encapsulated therein of step (C) are washed with an aqueous medium by centrifugation, gel filtration or dialysis.

20 4. The method of claim 2, wherein the organic solvent is selected from the group consisting of acetaldehyde, acetone, acetonitrile, allyl alcohol, allylamine, 2-amino-1-butanol, 1-aminoethanol, 2-aminoethanol, 2-amino-2-ethyl-1,3-propanediol, 2-amino-2-methyl-1-propanol, 3-aminopentane, N-(3-aminopropyl)morpholine, benzylamine, bis(2-ethoxyethyl) ether, bis(2-hydroxyethyl) ether, bis(2-hydropropyl) ether, bis(2-methoxyethyl) ether, 2-bromoethanol, meso-2,3-butanediol, 2-(2-butoxyethoxy)-ethanol, butylamine, sec-butylamine, tert-butylamine, 4-butyrolacetone, 2-chloroethanol, 1-chloro-2-propanol, 2-cyanoethanol, 3-cyanopyridine, cyclohexylamine, diethylamine,

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diethylenetriamine, N,N-diethylformamide, 1,2-dihydroxy-4-methylbenzene, N,N-dimethylacetamide, N,N-dimethylformamide, 2,6-dimethylmorpholine, 1,4-dioxane, 1,3-dioxolane, dipentaerythritol, ethanol, 2,3-epoxy-1-propanol, 2-ethoxyethanol, 2-(2-ethoxyethoxy)-ethanol, 2-(2-ethoxyethoxy)-ethyl acetate, ethylamine, 2-
5 (ethylamino)ethanol, ethylene glycol, ethylene oxide, ethylenimine, ethyl(-)-lactate, N-ethylmorpholine, ethyl-2-pyridine-carboxylate, formamide, furfuryl alcohol, furfurylamine, glutaric dialdehyde, glycerol, hexamethylphosphoramide, 2,5-hexanedione, hydroxyacetone, 2-hydroxyethyl-hydrazine, N-(2-hydroxyethyl)-morpholine, 4-hydroxy-4-methyl-2-pentanone, 5-hydroxy-2-pentanone, 2-
10 hydroxypropionitrile, 3-hydroxypropionitrile, 1-(2-hydroxy-1-propoxy)-2-propanol, isobutylamine, isopropylamine, 2-isopropylamino-ethanol, 2-mercaptoethanol, methanol, 3-methoxy-1-butanol, 2-methoxyethanol, 2-(2-methoxyethoxy)-ethanol, 1-methoxy-2-propanol, 2-(methylamino)-ethanol, 1-methylbutylamine, methylhydrazine, methyl hydroperoxide, 2-methylpyridine, 3-
15 methylpyridine, 4-methylpyridine, N-methylpyrrolidine, N-methyl-2-pyrrolidinone, morpholine, nicotine, piperidine, 1,2-propanediol, 1,3-propanediol, 1-propanol, 2-propanol, propylamine, propyleneimine, 2-propyn-1-ol, pyridine, pyrimidine, pyrrolidine, 2-pyrrolidinone and quinoxaline.

5. The method of claim 4, wherein the organic solvent is acetonitrile,
20 acetone or a C₁-C₃ alcohol.

6. The method of claim 5, wherein the organic solvent is methanol, ethanol, 1-propanol, 2-propanol, ethylene glycol or propylene glycol.

7. The method of claim 6, wherein the organic solvent is ethanol, 1-propanol or 2-propanol.

25 8. The method of claim 7, wherein the organic solvent is ethanol.

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9. The method of claim 4, wherein the organic solvent is acetone.

10. The method of claim 2, wherein aqueous medium U, aqueous medium V and/or aqueous medium W is an aqueous buffer.

11. The method of claim 2, wherein the gel or liquid and aqueous medium V are mixed in step (C) by adding aqueous medium V to the gel or the liquid containing gel particles.

12. The method of claim 2, wherein the gel or the liquid containing gel particles and aqueous medium V are mixed in step (C) by adding or infusing the gel or liquid into aqueous medium V.

13. The method of claim 2, wherein the at least one biologically active substance is a nucleic acid, pharmaceutical agent, diagnostic agent, protein, peptide, antigen, cytochrome C, transcription factor, cytokine or hapten.

14. The method of claim 13, wherein the at least one biologically active substance is a plasmid DNA.

15. The method of claim 14, wherein the plasmid DNA is up to about 20 kb in size.

16. The method of claim 15, wherein the plasmid DNA is of from about 0.5 kb to about 20 kb in size.

17. The method of claim 16, wherein the plasmid DNA is of about 1 kb to about 15 kb in size.

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18. The method of claim 17, wherein the plasmid DNA is of about 2 kb to about 10 kb in size.

19. The method of claim 18, wherein the plasmid DNA is of about 3 kb to about 7 kb in size.

5 20. The method of claim 2, wherein the at least one biologically active substance is selected from the group consisting of proteins and antigens structurally sensitive to dehydration.

21. The method of claim 20, wherein the proteins and antigens structurally sensitive to dehydration are tetanus toxoids.

10 22. The method of claim 2, wherein the at least one biologically active substance is at least one pharmaceutical agent selected from the group consisting of anti-neoplastic agents, anti-microbial agents, anti-viral agents, antihypertensive agents, anti-inflammatory agents, bronchodilators, local anesthetics and immunosuppressants.

15 23. The method of claim 22, wherein the at least one pharmaceutical agent is selected from the group consisting of anti-bacterial agents and anti-fungal agents.

20 24. The method of claim 22, wherein the at least one pharmaceutical agent is selected from the group consisting of anti-fungal agents and anti-neoplastic agents.

25. The method of claim 2, wherein the at least one biologically active substance is a bioreactive lipid.

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26. The method of claim 2, wherein the at least one biologically active substance is an antibody, enzyme or cytokine.

27. The method of claim 2, wherein the at least one biologically active substance is an RNA.

5 28. The method of claim 2, wherein the at least one biologically active substance is an oligonucleotide.

29. The method of claim 28, wherein the at least one biologically active substance is an oligonucleotide of about 5 to about 500 bases in size.

30. The method of claim 2, wherein the liposome of step (A) further
10 comprises a sterol.

31. The method of claim 30, wherein the sterol is cholesterol.

32. A method for preparing liposomes containing at least one biologically active substance encapsulated therein under mild conditions, said method comprising the following steps:

15 (A) (a) (i) providing liposomes, wherein the liposomes are prepared
by a method other than the instant method; and

(ii) mixing the liposomes of step (A)(a)(i) with the at least one biologically active substance;

(b) (i) providing liposomes in aqueous medium U, wherein the
20 liposomes are prepared by a method other than the instant method; and

(ii) mixing the liposomes of step (A)(b)(i) with the at least one biologically active substance;

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(c) (i) providing liposomes, wherein the liposomes are prepared by a method other than the instant method; and

(ii) mixing the liposomes of step (A)(c)(i) with aqueous medium U and the at least one biologically active substance;

5 (d) (i) providing liposomes in aqueous medium U, wherein the liposomes are prepared by a method other than the instant method; and

(ii) mixing the liposomes of step (A)(d)(i) with aqueous medium U and the at least one biologically active substance;

(e) forming liposomes in the presence of the at least one biologically active substance by a method other than the instant method;

10 (f) providing liposomes containing the at least one biologically active substance, wherein the liposomes are prepared by a method other than the instant method; or

(g) providing liposomes, wherein the liposomes are prepared by a method other than the instant method;

15 (B) (a) mixing the product of step (A)(b), (A)(c) or (A)(d) with a water-miscible organic solvent to form a gel or a liquid containing gel particles;

(b) mixing the product of step (A)(a), (A)(e) or (A)(f) with aqueous medium U and a water-miscible organic solvent to form a gel or a liquid containing gel particles;

20 (c) mixing the product of step (A)(g) with aqueous medium U, a water-miscible organic solvent and the at least one biologically active substance to form a gel or a liquid containing gel particles; or

(d) mixing the product of step (A)(g) with aqueous medium U and a water-miscible organic solvent to form a gel or a liquid containing gel particles;

25 and thereafter

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- (C) (a) mixing the gel or liquid containing gel particles of step (B)(a), (B)(b) or (B)(c) with aqueous medium V to directly form the liposomes containing the at least one biologically active substance encapsulated therein,
- 5 (b) (i) mixing the gel or liquid containing gel particles of step (B)(a), (B)(b) or (B)(c) with aqueous medium V to form a curd or curdy substance; and
- (ii) mixing the curd or curdy substance with aqueous medium W to directly form the liposomes containing the at least one biologically active substance encapsulated therein;
- 10 (c) (i) cooling the gel or liquid containing gel particles of step (B)(a), (B)(b) or (B)(c) to form a waxy substance;
- (ii) mixing the waxy substance with aqueous medium W to directly form the liposomes containing the at least one biologically active substance encapsulated therein;
- 15 (d) mixing the gel or liquid containing gel particles of step (B)(d) with aqueous medium V and the at least one biologically active substance to directly form the liposomes containing the at least one biologically active substance encapsulated therein,
- 20 (e) (i) mixing the gel or liquid containing gel particles of step (B)(d) with aqueous medium V and the at least one biologically active substance to form a curd or curdy substance; and
- (ii) mixing the curd or curdy substance with aqueous medium W to directly form the liposomes containing the at least one biologically active substance encapsulated therein;
- 25 (f) (i) mixing the gel or liquid containing gel particles of step (B)(d) with aqueous medium V to form a curd or curdy substance; and
- (ii) mixing the curd or curdy substance with aqueous medium W and the at least one biologically active substance to directly form the

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liposomes containing the at least one biologically active substance encapsulated therein; or

(g) (i) cooling the gel or liquid containing gel particles of step (B)(d) to form a waxy substance;

5 (ii) mixing the waxy substance with aqueous medium W and the at least one biologically active substance to directly form the liposomes containing the at least one biologically active substance encapsulated therein;

wherein aqueous media U, V and W are the same or different and a phospholipid content of the gel or the liquid containing gel particles is not 15 to 30% by weight of the gel or the liquid containing gel particles.

33. The method of claim 32, wherein step (C)(a) or step (C)(b)(i) is conducted by mixing the gel or liquid with aqueous medium V and the at least one biologically active substance, and/or step (C)(b)(ii) is conducted by mixing the curd or curdy substance with aqueous medium W and the at least one biologically
15 active substance.

34. The method of claim 32, wherein the liposomes containing the at least one biologically active substance encapsulated therein of step (C) are washed with an aqueous medium by centrifugation, gel filtration or dialysis.

35. The method of claim 32, wherein the organic solvent is selected from
20 the group consisting of acetaldehyde, acetone, acetonitrile, allyl alcohol, allylamine, 2-amino-1-butanol, 1-aminoethanol, 2-aminoethanol, 2-amino-2-ethyl-1,3-propanediol, 2-amino-2-methyl-1-propanol, 3-aminopentane, N-(3-aminopropyl)morpholine, benzylamine, bis(2-ethoxyethyl) ether, bis(2-hydroxyethyl) ether, bis(2-hydropropyl) ether, bis(2-methoxyethyl) ether, 2-bromoethanol, meso-2,3-butanediol, 2-(2-butoxyethoxy)-ethanol, butylamine, sec-
25 butylamine, tert- butylamine, 4-butyrolacetone, 2-chloroethanol, 1-chloro-2-

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propanol, 2-cyanoethanol, 3-cyanopyridine, cyclohexylamine, diethylamine, diethylenetriamine, N,N-diethylformamide, 1,2-dihydroxy-4-methylbenzene, N,N-dimethylacetamide, N,N-dimethylformamide, 2,6-dimethylmorpholine, 1,4-dioxane, 1,3-dioxolane, dipentaerythritol, ethanol, 2,3-epoxy-1-propanol, 2-ethoxyethanol, 5 2-(2-ethoxyethoxy)-ethanol, 2-(2-ethoxyethoxy)-ethyl acetate, ethylamine, 2-(ethylamino)ethanol, ethylene glycol, ethylene oxide, ethylenimine, ethyl(-)-lactate, N-ethylmorpholine, ethyl-2-pyridine-carboxylate, formamide, furfuryl alcohol, furfurylamine, glutaric dialdehyde, glycerol, hexamethylphosphoramide, 2,5-hexanedione, hydroxyacetone, 2-hydroxyethylhydrazine, N-(2-hydroxyethyl)-10 morpholine, 4-hydroxy-4-methyl-2-pentanone, 5-hydroxy-2-pentanone, 2-hydroxypropionitrile, 3-hydroxypropionitrile, 1-(2-hydroxy-1-propoxy)-2-propanol, isobutylamine, isopropylamine, 2-isopropylamino-ethanol, 2-mercaptoethanol, methanol, 3-methoxy-1-butanol, 2-methoxyethanol, 2-(2-methoxyethoxy)-ethanol, 1-methoxy-2-propanol, 2-(methylamino)-ethanol, 1-15 methylbutylamine, methylhydrazine, methyl hydroperoxide, 2-methylpyridine, 3-methylpyridine, 4-methylpyridine, N-methylpyrrolidine, N-methyl-2-pyrrolidinone, morpholine, nicotine, piperidine, 1,2-propanediol, 1,3-propanediol, 1-propanol, 2-propanol, propylamine, propyleneimine, 2-propyn-1-ol, pyridine, pyrimidine, pyrrolidine, 2-pyrrolidinone and quinoxaline.

20 36. The method of claim 35, wherein the organic solvent is methanol, ethanol, 1-propanol, 2-propanol, ethylene glycol or propylene glycol.

37. The method of claim 36, wherein the organic solvent is ethanol, 1-propanol or 2-propanol.

38. The method of claim 37, wherein the organic solvent is ethanol.

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39. The method of claim 35, wherein the organic solvent is acetonitrile or acetone.

40. The method of claim 32, wherein aqueous medium U, aqueous medium V and/or aqueous medium W is an aqueous buffer.

5 41. The method of claim 32, wherein the gel or liquid and aqueous medium V are mixed in step (C) by adding aqueous medium V to the gel or the liquid containing gel particles.

 42. The method of claim 32, wherein the gel or the liquid containing gel particles and aqueous medium V are mixed in step (C) by adding or infusing the
10 gel or liquid into aqueous medium V.

 43. The method of claim 32, wherein the at least one biologically active substance is a nucleic acid, protein, peptide, enzyme, cytochrome C, transcription factor, cytokine, antigen, hapten, pharmaceutical agent or diagnostic agent.

 44. The method of claim 43, wherein the at least one biologically active
15 substance is a plasmid DNA.

 45. The method of claim 44, wherein the plasmid DNA is up to about 20 kb in size.

 46. The method of claim 45, wherein the plasmid DNA is of from about 0.5 kb to about 20 kb in size.

20 47. The method of claim 46, wherein the plasmid DNA is of about 1 kb to about 15 kb in size.

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48. The method of claim 47, wherein the plasmid DNA is of about 2 kb to about 10 kb in size.

49. The method of claim 48, wherein the plasmid DNA is of about 3 kb to about 7 kb in size.

5 50. The method of claim 32, wherein the at least one biologically active substance is selected from the group consisting of proteins and antigens structurally sensitive to dehydration.

51. The method of claim 50, wherein the at least one biologically active substance is a tetanus toxoid.

10 52. The method of claim 32, wherein the at least one biologically active substance is at least one pharmaceutical agent selected from the group consisting of anti-neoplastic agents, anti-microbial agents, anti-viral agents, antihypertensive agents, anti-inflammatory agents, bronchodilators, local anesthetics and immunosuppressants.

15 53. A method for preparing liposomes containing at least one biologically active substance encapsulated therein under mild conditions, said method comprising the following steps:

(A) (a) (i) providing liposomes, wherein the liposomes are prepared by a method other than the instant method; and

20 (ii) mixing the liposomes of step (A)(a)(i) with the at least one biologically active substance;

(b) (i) providing liposomes in aqueous medium U, wherein the liposomes are prepared by a method other than the instant method; and

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(ii) mixing the liposomes of step (A)(b)(i) with the at least one biologically active substance;

(c) (i) providing liposomes, wherein the liposomes are prepared by a method other than the instant method; and

5 (ii) mixing the liposomes of step (A)(c)(i) with aqueous medium U and the at least one biologically active substance;

(d) (i) providing liposomes in aqueous medium U, wherein the liposomes are prepared by a method other than the instant method; and

10 (ii) mixing the liposomes of step (A)(d)(i) with aqueous medium U and the at least one biologically active substance;

(e) forming liposomes in the presence of the at least one biologically active substance by a method other than the instant method; or

15 (f) forming liposomes in aqueous medium U in the presence of the at least one biologically active substance by a method other than the instant method;

(B) (a) mixing the product of step (A)(b), (A)(c), (A)(d) or (A)(f) with a water-miscible organic solvent to form a gel or a liquid containing gel particles; or

20 (b) mixing the product of step (A)(a) or (A)(e) with aqueous medium U and a water-miscible organic solvent to form a gel or a liquid containing gel particles; and thereafter

(C) (a) mixing the gel or liquid containing gel particles with aqueous medium V to directly form the liposomes containing the at least one biologically active substance encapsulated therein, or

25 (b) (i) mixing the gel or liquid containing gel particles with aqueous medium V to form a curd or curdy substance; and

(ii) mixing the curd or curdy substance with aqueous medium W to directly form the liposomes containing the at least one biologically active substance encapsulated therein,

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wherein aqueous media U, V and W are the same or different and the gel or the liquid containing gel particles contain no hydrating agent.

54. The method of claim 53, wherein step (C)(a) or step (C)(b)(i) is conducted by mixing the gel or liquid with aqueous medium V and the at least one
5 biologically active substance, and/or step (C)(b)(ii) is conducted by mixing the curd or curdy substance with aqueous medium W and the at least one biologically active substance.

55. The method of claim 53, wherein the liposomes containing the at least one biologically active substance encapsulated therein of step (C) are washed with
10 an aqueous medium by centrifugation, gel filtration or dialysis.

56. The method of claim 53, wherein the organic solvent is selected from the group consisting of acetaldehyde, acetone, acetonitrile, allyl alcohol, allylamine, 2-amino-1-butanol, 1-aminoethanol, 2-aminoethanol, 2-amino-2-ethyl-1,3-propanediol, 2-amino-2-methyl-1-propanol, 3-aminopentane, N-(3-aminopropyl)morpholine, benzylamine, bis(2-ethoxyethyl) ether, bis(2-hydroxyethyl) ether, bis(2-hydropropyl) ether, bis(2-methoxyethyl) ether, 2-bromoethanol, meso-2,3-butanediol, 2-(2-butoxyethoxy)-ethanol, butylamine, sec-butylamine, tert-butylamine, 4-butyrolactone, 2-chloroethanol, 1-chloro-2-propanol, 2-cyanoethanol, 3-cyanopyridine, cyclohexylamine, diethylamine,
15 diethylenetriamine, N,N-diethylformamide, 1,2-dihydroxy-4-methylbenzene, N,N-dimethylacetamide, N,N-dimethylformamide, 2,6-dimethylmorpholine, 1,4-dioxane, 1,3-dioxolane, dipentaerythritol, ethanol, 2,3-epoxy-1-propanol, 2-ethoxyethanol, 2-(2-ethoxyethoxy)-ethanol, 2-(2-ethoxyethoxy)-ethyl acetate, ethylamine, 2-(ethylamino)ethanol, ethylene glycol, ethylene oxide, ethylenimine, ethyl(-)-lactate, N-ethylmorpholine, ethyl-2-pyridine-carboxylate, formamide, furfuryl
20 alcohol, furfurylamine, glutaric dialdehyde, glycerol, hexamethylphosphoramide,

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2,5-hexanedione, hydroxyacetone, 2-hydroxyethyl-hydrazine, N-(2-hydroxyethyl)-morpholine, 4-hydroxy-4-methyl-2-pentanone, 5-hydroxy-2-pentanone, 2-hydroxypropionitrile, 3-hydroxypropionitrile, 1-(2-hydroxy-1-propoxy)-2-propanol, isobutylamine, isopropylamine, 2-isopropylamino-ethanol, 2-mercaptoethanol, methanol, 3-methoxy-1-butanol, 2-methoxyethanol, 2-(2-methoxyethoxy)-ethanol, 1-methoxy-2-propanol, 2-(methylamino)-ethanol, 1-methylbutylamine, methylhydrazine, methyl hydroperoxide, 2-methylpyridine, 3-methylpyridine, 4-methylpyridine, N-methylpyrrolidine, N-methyl-2-pyrrolidinone, morpholine, nicotine, piperidine, 1,2-propanediol, 1,3-propanediol, 1-propanol, 2-propanol, propylamine, propyleneimine, 2-propyn-1-ol, pyridine, pyrimidine, pyrrolidine, 2-pyrrolidinone and quinoxaline.

57. The method of claim 56, wherein the organic solvent is acetonitrile, acetone or a C₁-C₃ alcohol.

58. The method of claim 57, wherein the organic solvent is methanol, ethanol, 1-propanol, 2-propanol, ethylene glycol or propylene glycol.

59. The method of claim 58, wherein the organic solvent is ethanol, 1-propanol or 2-propanol.

60. The method of claim 59, wherein the organic solvent is ethanol.

61. The method of claim 56, wherein the organic solvent is acetone.

62. The method of claim 53, wherein aqueous medium U, aqueous medium V and/or aqueous medium W is an aqueous buffer.

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63. The method of claim 2, wherein the liposomes of step (A) comprise at least one fusogenic lipid.

64. The method of claim 63, wherein the at least one fusogenic lipid is selected from the group consisting of N-acyl phosphatidylethanolamine.

5 65. The method of claim 64, wherein the at least one fusogenic lipid is selected from the group consisting of N-decanoyl phosphatidylethanolamine, N-dodecanoyl phosphatidylethanolamine and N-tetradecanoyl phosphatidylethanolamine.

10 66. The method of claim 65, wherein the at least one fusogenic lipid is selected from the group consisting of N-dodecanoyl phosphatidylethanolamine.

67. The method of claim 32, wherein the liposomes of step (A) comprise at least one fusogenic lipid.

68. The method of claim 67, wherein the at least one fusogenic lipid is selected from the group consisting of N-acyl phosphatidylethanolamine.

15 69. The method of claim 68, wherein the at least one fusogenic lipid is selected from the group consisting of N-decanoyl phosphatidylethanolamine, N-dodecanoyl phosphatidylethanolamine and N-tetradecanoyl phosphatidylethanolamine.

20 70. The method of claim 69, wherein the at least one fusogenic lipid is selected from the group consisting of N-dodecanoyl phosphatidylethanolamine.

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71. The method of claim 53, wherein the liposomes of step (A) comprise at least one fusogenic lipid.

72. The method of claim 71, wherein the at least one fusogenic lipid is selected from the group consisting of N-acyl phosphatidylethanolamine.

5 73. The method of claim 72, wherein the at least one fusogenic lipid is selected from the group consisting of N-decanoyl phosphatidylethanolamine, N-dodecanoyl phosphatidylethanolamine and N-tetradecanoyl phosphatidylethanolamine.

10 74. The method of claim 73, wherein the at least one fusogenic lipid is selected from the group consisting of N-dodecanoyl phosphatidylethanolamine.

15 75. The method of claim 2, wherein aqueous medium V is mixed in increments with the gel or the liquid containing gel particles in step (C), wherein the increments are up to about 100% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

76. The method of claim 75, wherein the increments are up to about 80% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

20 77. The method of claim 76, wherein the increments are up to about 60% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

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78. The method of claim 77, wherein the increments are up to about 40% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

5 79. The method of claim 78, wherein the increments are up to about 20% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

80. The method of claim 79, wherein the increments are up to about 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

10 81. The method of claim 80, wherein the increments are up to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

15 82. The method of claim 81, wherein the increments are up to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

83. The method of claim 82, wherein the increments are up to about 0.5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

20 84. The method of claim 83, wherein the increments are up to about 0.1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

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85. The method of claim 80, wherein the increments are from about 0.001% to about 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

5 86. The method of claim 85, wherein the increments are from about 0.001% to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

87. The method of claim 86, wherein the increments are from about 0.001% to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

10 88. The method of claim 32, wherein aqueous medium V is mixed in increments with the gel or the liquid containing gel particles in step (C), wherein the increments are up to about 100% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

15 89. The method of claim 88, wherein the increments are up to about 80% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

20 90. The method of claim 89, wherein the increments are up to about 60% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

91. The method of claim 90, wherein the increments are up to about 40% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

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92. The method of claim 91, wherein the increments are up to about 20% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

5 93. The method of claim 92, wherein the increments are up to about 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

94. The method of claim 93, wherein the increments are up to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

10 95. The method of claim 94, wherein the increments are up to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

15 96. The method of claim 95, wherein the increments are up to about 0.5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

97. The method of claim 96, wherein the increments are up to about 0.1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

20 98. The method of claim 93, wherein the increments are from about 0.001% to about 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

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99. The method of claim 98, wherein the increments are from about 0.001% to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

100. The method of claim 99, wherein the increments are from about
5 0.001% to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

101. The method of claim 53, wherein aqueous medium V is mixed in increments with the gel or the liquid containing gel particles in step (C), wherein the increments are up to about 100% of the weight of the gel or the liquid
10 containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

102. The method of claim 101, wherein the increments are up to about 80% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

103. The method of claim 102, wherein the increments are up to about
15 60% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

104. The method of claim 103, wherein the increments are up to about
40% of the weight of the gel or the liquid containing gel particles before the gel or
20 the liquid is mixed with any aqueous medium V.

105. The method of claim 104, wherein the increments are up to about 20% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

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106. The method of claim 105, wherein the increments are up to about 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

5 107. The method of claim 106, wherein the increments are up to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

108. The method of claim 107, wherein the increments are up to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

10 109. The method of claim 108, wherein the increments are up to about 0.5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

110. The method of claim 109, wherein the increments are up to about 0.1% of the weight of the gel or the liquid containing gel particles before the gel
15 or the liquid is mixed with any aqueous medium V.

111. The method of claim 106, wherein the increments are from about 0.001% to about 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

112. The method of claim 111, wherein the increments are from about
20 0.001% to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

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113. The method of claim 112, wherein the increments are from about 0.001% to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

5 114. The method of claim 53, wherein the at least one biologically active substance is selected from the group consisting of proteins and antigens structurally sensitive to dehydration.

115. The method of claim 114, wherein the at least one biologically active substance is a tetanus toxoid.

10 116. The method of claim 2, wherein the liposomes provided in step (A)(a), (A)(b), (A)(c), (A)(d) or (A)(f) comprise at least one charged lipid, the liposomes in step (A)(e) are formed in the presence of at least one charged lipid and the at least one biologically active substance, or at least one charged lipid is added in step (B), wherein the at least one charged lipid is a lipid having a net negative or positive charge.

15 117. The method of claim 116, wherein the at least one charged lipid is selected from the group consisting of N-acyl phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and phosphatidic acid.

20 118. The method of claim 116, wherein the at least one charged lipid is liposome forming.

119. The method of claim 32, wherein the liposomes provided in step (A)(a), (A)(b), (A)(c), (A)(d) or (A)(f) comprise at least one charged lipid, the liposomes in step (A)(e) are formed in the presence of at least one charged lipid

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and the at least one biologically active substance, or at least one charged lipid is added in step (B), wherein the at least one charged lipid is a lipid having a net negative or positive charge.

120. The method of claim 119, wherein the at least one charged lipid is
5 selected from the group consisting of N-acyl phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and phosphatidic acid.

121. The method of claim 119, wherein the at least one charged lipid is liposome forming.

10 122. The method of claim 53, wherein the liposomes provided in step (A)(a), (A)(b), (A)(c), (A)(d) or (A)(f) comprise at least one charged lipid, the liposomes in step (A)(e) are formed in the presence of at least one charged lipid and the at least one biologically active substance, or at least one charged lipid is added in step (B), wherein the at least one charged lipid is a lipid having a net
15 negative or positive charge.

123. The method of claim 122, wherein the at least one charged lipid is selected from the group consisting of N-acyl phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and phosphatidic acid.

20 124. The method of claim 123, wherein the at least one charged lipid is liposome forming.

125. The method of claim 2, wherein the amount of lipid in the gel or the liquid containing gel particles of step (B) ranges from 1% by weight of the gel or

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the liquid containing gel particles to the hydration limit of the lipid in water, wherein the "hydration limit" is the maximum amount of lipid in a given amount of water that would keep the lipid in a liposomal state.

126. The method of claim 2, wherein the amount of lipid in the gel or the liquid containing gel particles of step (B) ranges from about 5% to about 95% by weight of the gel or the liquid containing gel particles.

127. The method of claim 126, wherein said amount of lipid ranges from about 10% to about 95% by weight of the gel or the liquid containing gel particles.

128. The method of claim 127, wherein said amount of lipid ranges from about 15% to about 95% by weight of the gel or the liquid containing gel particles.

129. The method of claim 128, wherein said amount of lipid ranges from about 20% to about 95% by weight of the gel or the liquid containing gel particles.

130. The method of claim 129, wherein said amount of lipid ranges from about 30% to about 95% by weight of the gel or the liquid containing gel particles.

131. The method of claim 130, wherein said amount of lipid ranges from about 40% to about 95% by weight of the gel or the liquid containing gel particles.

132. The method of claim 131, wherein said amount of lipid ranges from about 50% to about 95% by weight of the gel or the liquid containing gel particles.

133. The method of claim 132, wherein said amount of lipid ranges from about 60% to about 95% by weight of the gel or the liquid containing gel particles.

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134. The method of claim 133, wherein said amount of lipid ranges from about 70% to about 95% by weight of the gel or the liquid containing gel particles.

135. The method of claim 32, wherein the amount of lipid in the gel or the liquid containing gel particles of step (B) ranges from 1% by weight of the gel or
5 the liquid containing gel particles to the hydration limit of the lipid in water, wherein the "hydration limit" is the maximum amount of lipid in a given amount of water that would keep the lipid in a liposomal state.

136. The method of claim 32, wherein the amount of lipid in the gel or the liquid containing gel particles of step (B) ranges from about 5% to about 95% by
10 weight of the gel or the liquid containing gel particles.

137. The method of claim 136, wherein said amount of lipid ranges from about 10% to about 95% by weight of the gel or the liquid containing gel particles.

138. The method of claim 137, wherein said amount of lipid ranges from about 15% to about 95% by weight of the gel or the liquid containing gel particles.

15 139. The method of claim 138, wherein said amount of lipid ranges from about 20% to about 95% by weight of the gel or the liquid containing gel particles.

140. The method of claim 139, wherein said amount of lipid ranges from about 30% to about 95% by weight of the gel or the liquid containing gel particles.

20 141. The method of claim 140, wherein said amount of lipid ranges from about 40% to about 95% by weight of the gel or the liquid containing gel particles.

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142. The method of claim 141, wherein said amount of lipid ranges from about 50% to about 95% by weight of the gel or the liquid containing gel particles.

143. The method of claim 142, wherein said amount of lipid ranges from about 60% to about 95% by weight of the gel or the liquid containing gel particles.

5 144. The method of claim 143, wherein said amount of lipid ranges from about 70% to about 95% by weight of the gel or the liquid containing gel particles.

10 145. The method of claim 53, wherein the amount of lipid in the gel or the liquid containing gel particles of step (B) ranges from 1% by weight of the gel or the liquid containing gel particles to the hydration limit of the lipid in water, wherein the "hydration limit" is the maximum amount of lipid in a given amount of water that would keep the lipid in a liposomal state.

146. The method of claim 53, wherein the amount of lipid in the gel or the liquid containing gel particles of step (B) ranges from about 5% to about 95% by weight of the gel or the liquid containing gel particles.

15 147. The method of claim 146, wherein said amount of lipid ranges from about 10% to about 95% by weight of the gel or the liquid containing gel particles.

148. The method of claim 147, wherein said amount of lipid ranges from about 15% to about 95% by weight of the gel or the liquid containing gel particles.

20 149. The method of claim 148, wherein said amount of lipid ranges from about 20% to about 95% by weight of the gel or the liquid containing gel particles.

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150. The method of claim 149, wherein said amount of lipid ranges from about 30% to about 95% by weight of the gel or the liquid containing gel particles.

151. The method of claim 150, wherein said amount of lipid ranges from about 40% to about 95% by weight of the gel or the liquid containing gel particles.

5 152. The method of claim 151, wherein said amount of lipid ranges from about 50% to about 95% by weight of the gel or the liquid containing gel particles.

153. The method of claim 152, wherein said amount of lipid ranges from about 60% to about 95% by weight of the gel or the liquid containing gel particles.

10 154. The method of claim 153, wherein said amount of lipid ranges from about 70% to about 95% by weight of the gel or the liquid containing gel particles.